

## WHAT IS CLAIMED IS:

1. A process for production of plasmid DNA comprising:
  - (a) selecting a highly productive clonal subtype of a strain of *E. coli* harboring a DNA plasmid, wherein a highly productive clonal subtype exhibits a higher plasmid copy number per cell in comparison to non-selected, transformed *E. coli* clonal subtypes of the same strain; and,
    - (b) cultivating said highly productive clonal subtype with fed-batch fermentation in chemically-defined medium.
- 10 2. A process of claim 1, wherein said selection in step (a) comprises:
  - (a) a first selection step wherein potential highly productive clonal subtypes of a strain of *E. coli* harboring a DNA plasmid are isolated; and,
  - (b) a second selection step wherein said potential highly productive clonal subtypes isolated in step (a) are tested to determine which of said clonal subtypes are highly productive.
- 15 3. A process of claim 2, wherein said strain of *E. coli* is DH5.
4. A process of claim 2, wherein colonies of said potential highly productive clonal subtypes of step (a) are phenotypically gray on blood agar.
- 20 5. A process of claim 4, wherein said blood agar is incubated for about 48 hours at about 30°C.
6. A process of claim 2, wherein colonies of said potential highly productive clonal subtypes of step (a) are phenotypically cream on chemically-defined agar medium after incubating said *E. coli* until a population of both cream-colored colonies and cream-colored colonies with brown, bulls-eyed centers have formed.
- 25 7. A process of claim 6, wherein said chemically-defined agar medium is DME-P5 agar medium.
- 30 8. A process of claim 7, wherein said chemically-defined agar medium is incubated at about 37°C for about 5 days.

9. A process of claim 1, wherein said chemically-defined medium in step (b) comprises a medium selected from the group consisting of Medium C, Medium D, Medium E, Medium F and Medium G.

5 10. A process of claim 1, wherein said highly productive clonal subtypes of a strain of *E. coli* are cultivated in step (b) on an industrial scale in a chemically-defined medium.

10 11. A process of claim 2, wherein said potential highly productive clonal subtypes are tested in step (b) in a small-scale fermentation system to determine which clonal subtypes are highly productive.

12. A process of claim 11, wherein said small-scale fermentation system of step (b) used to test productivity of the potential highly productive clonal subtypes is a shake flask fermentation system using chemically-defined cultivation medium.

15 13. A process of claim 12, wherein said chemically-defined medium comprises DME-B12 medium.

20 14. A process of claim 12, wherein a solution is continuously fed to a shake flask containing said potential highly productive clonal subtypes when said clonal subtypes are in mid-logarithmic phase of growth.

15. A process of claim 14, wherein said feed solution comprises about 4.6% glycerol (v/v) and about 2.9% monosodium glutamate (w/v).

25 16. A process of claim 10, wherein said chemically-defined medium comprises a medium selected from the group consisting of Medium C, Medium D, Medium E, Medium F and Medium G.

17. A process of claim 10, wherein said cultivation step comprises at least one production stage fermentation phase.

30 18. A process of claim 17, wherein a solution is continuously fed to a production stage fermentor containing a highly productive clonal subtype when said clonal subtype is in mid-logarithmic phase of growth.

19. A process of claim 18, wherein said feed solution comprises about 50% glycerol (v/v) and about 25% monosodium glutamate (w/v).

20. A process of claim 18, wherein said feed solution comprises about 60% glycerol (v/v).

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21. A method for selecting a highly productive clonal subtype of a strain of *E. coli* for plasmid DNA production comprising the steps of:

(a) purifying colonies of a strain of *E. coli* harboring a DNA plasmid that are phenotypically gray on blood agar, wherein a gray-colored colony represents a potential highly productive clonal subtype; and,

(b) testing productivity of said potential highly productive clonal subtypes, wherein a highly productive clonal subtype exhibits a higher plasmid copy number per cell in comparison to similarly tested *E. coli* clonal subtypes of the same strain.

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22. A process of claim 21, wherein said strain of *E. coli* is DH5.

23. A method of claim 21, wherein said blood agar in step (a) is incubated for about 48 hours at about 30°C.

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24. A method of claim 21, wherein the productivity of said potential highly productive clonal subtypes of step (b) is determined after cultivating said clonal subtypes in a shake flask fermentation system in chemically-defined medium.

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25. A method of claim 24, wherein said chemically-defined medium comprises DME-B12 medium.

26. A method of claim 24, wherein a solution is continuously fed to a shake flask when the potential highly productive clonal subtypes are in mid-logarithmic phase of growth.

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27. A method of claim 26, wherein said feed solution comprises about 4.6% glycerol (v/v) and about 2.9 % monosodium glutamate (w/v).

28. A method for selecting a highly productive clonal subtype of a strain of *E. coli* for plasmid DNA production comprising the steps of:

(a) incubating a strain of *E. coli* harboring a DNA plasmid on chemically-defined agar medium until a population of both cream-colored colonies and cream-colored colonies with brown, bulls-eye centers have formed;

5 (b) purifying said cream-colored colonies from step (a), wherein a cream-colored colony represents a potential highly productive clonal subtype;

(c) testing productivity of said potential highly productive clonal subtypes, wherein a highly productive clonal subtype exhibits a higher plasmid copy number per cell in comparison to similarly tested *E. coli* clonal subtypes of the same strain.

10 29. A process of claim 28, wherein said strain of *E. coli* is DH5.

30. A method of claim 28, wherein said chemically-defined medium agar described in step (a) is incubated at about 37°C for about 5 days.

15 31. A method of claim 28, wherein the productivity of said potential highly productive clonal subtypes of step (c) is determined after cultivating said clonal subtypes in a shake flask fermentation system in chemically-defined medium.

20 32. A method of claim 31, wherein said chemically-defined medium comprises DME-B12 medium.

33. A method of claim 31, wherein a solution is continuously fed to a shake flask when the potential highly productive clonal subtypes are in mid-logarithmic phase of growth.

25 34. A method of claim 33, wherein said feed solution comprises about 4.6% glycerol (v/v) and about 2.9 % monosodium glutamate (w/v).